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Nocturnalism in *Aplysia oculifera* (Adams & Reeve, 1850): An Avoidance Behavior Minimizing Exposure to Ultraviolet Radiation?

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Abstract. Circadian activity patterns and preferred location were recorded under experimental conditions in two groups of the sea hare *Aplysia oculifera,* one exposed to direct sunlight (group L) and the other placed in the shade (group S). Observations were made several times during the day and night for 20 days. Activities (resting, feeding, crawling, copulating and spawning) and locations (buried in the sand, under stones, on the substrate and on the algae) were recorded. Data were pooled into four time blocks (midnight to 06:00, 06:01 to 12:00, 12:01 to 18:00, and 18:01 to midnight). Sea hares of group L showed distinct nocturnal activity pattern, feeding at night when most of them were found on the algae, and resting during the day when they were located mostly under the stones. In group S no significant differences were detected between day and night activities or preferred location. The results show that the nocturnal activity pattern in the sea hare *A. oculifera* is governed by an external factor, probably direct sunlight, rather than an internal cue. It is suggested that *A. oculifera* is nocturnally active to avoid exposure to direct ultraviolet radiation.

INTRODUCTION

Aplysia oculifera (Adams & Reeve, 1850) is a widespread sea hare species, distributed in tropical and subtropical regions from the eastern coast of Africa, including the Red Sea, through the Indo-Pacific Ocean to the islands of Hawaii. *Aplysia oculifera* is a medium-sized sea hare (5–100 g live body weight), which dwells on shallow rocky shores, and feeds exclusively on macroalgae, mainly the green algae *Ulva* spp. and *Enteromorpha* spp (Plaut, 1993; Plaut et al., 1998).

Among sea hares of the genus *Aplysia,* some species are nocturnal and others diurnal (Susswein et al., 1984; Carefoot, 1987, 1989; Carefoot & Taylor, 1988). It is as yet unknown whether the cue for these patterns of activity is endogenous or exogenous, and what advantages sea hares gain by being either nocturnal or diurnal.

Carefoot & Taylor (1988) listed several possible reasons for nocturnalism in *A. dactylomela* Rang, 1828 (which ecologically resembles *A. oculifera*), such as avoiding predators, avoiding diurnal interspecific or conspecific competitors, and intolerance to light, particularly ultraviolet wavelengths. They strongly rejected avoidance of predation and competition as possible reasons because (1) *Aplysia* has few or no predators (Carefoot, 1987), and instances of predation known for *A. dactylomela* are isolated events of apparent opportunism involving only juveniles and eggs being eaten (Willan, 1979). In addition, the defensive repertoire of adult *Aplysia* includes purple ink and opaline gland secretions, unpalatable skin, and toxic digestive gland; (2) most interspecific competitors seem to show no effect on algae abundance or are nocturnal as well (e.g., Hobson, 1974); and (3) conspecific competition will not be avoided if the entire population is nocturnal. These same arguments apply also to *A. oculifera* in the Gulf of Eilat (Plaut, 1993). Carefoot & Taylor (1988), and Carefoot (1989) also rejected the avoidance of ultraviolet radiation as a possible reason because (1) light is unlikely to have operated as a primary force leading to the evolution of nocturnalism; (2) *Aplysia* makes no obvious attempt to protect its eggs from direct sunlight; and (3) other tropical and subtropical sea hares inhabiting shallow water are diurnal.

They suggested that there may be a metabolic advantage to splitting the day into two phases of activity (feeding and resting) but could not explain the advantage of nocturnalism versus diurnalism or vice versa.

Surveys conducted in the Gulf of Eilat (Aqaba) show that *A. oculifera* is mostly nocturnal (Plaut, 1993; Plaut et al., 1998). Its nighttime activities include feeding, crawling, copulating, and spawning. During the daytime, sea hares mostly rest either under stones or buried in the sand. Contrary to this general observation, in several cases, *A. oculifera* in the Gulf of Eilat was observed feeding, crawling, copulating, and spawning during daytime when algae were very abundant and when it was found at a depth below 2 m (Plaut, 1993).

In this study I examined the question of whether the nocturnalism of *A. oculifera* is a response to external cue, e.g., direct radiation (sunlight), or whether it is governed by internal cues. I followed the locations and activities of two groups of *A. oculifera* under outdoor experimental

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conditions during daytime and nighttime, one exposed to direct natural sunlight and the other under shade.

MATERIALS AND METHODS

Newly recruited (>1 g live body weight at the beginning of the settlement season) sea hares were collected at several sites in the northern Gulf of Eilat (Aqaba) in February 1990. They were kept at the Interuniversity Institute for Marine Sciences of Eilat in an open circulated seawater system. Experiments were initiated within 1–2 days after collection. Two groups of 20 individuals of similar size (0.8–1.9 g body live mass) were reared in two uncovered 30 L glass aquaria (50 × 25 × 25 cm length, width, and height, respectively). Both aquaria were placed outdoors. One was in the shade (group S), under a roof and with one of its sides a wall facing west, and the other three sides left open; the other was under direct natural sunlight (group L). Each aquarium was supplied with continuous water flow (2–3 L min^{-1}) to maintain similar temperatures (day water temperature, 26 ± 1 °C and night temperature 22 ± 1 °C with no significant differences between the two aquaria). Each aquarium contained a layer of 3–4 cm sand from the sea hares' natural habitat, and two stones, 10–13 cm in diameter, that were placed as shelters for the sea hares. Food (*Ulva* sp.) was provided in amounts sufficient for *ad libitum* feeding, but at small rations and in small pieces so as to prevent sea hares from using them as shelters against sunlight. Location of the sea hares and their activities were recorded randomly four to six times daily (day and night) during 20 days. Four areas were defined in the aquaria for the location of the sea hares: (1) under stone; (2) buried in the sand; (3) on the substrate (including sand, stone, and aquarium walls); and (4) on the algae. Five different activities were defined (after Carefoot, 1989): (1) resting (inactive); (2) feeding (biting or chewing); (3) crawling (moving around without feeding, spawning or copulating); (4) spawning (laying eggs); and (5) copulating. In several cases, when sea hares were observed conducting more than one type of activity simultaneously, (e.g., feeding and copulating, copulating and spawning), all activities were recorded for each individual. The results were treated for each location or activity as percentages of all the sea hares in the aquarium.

Although the objective of this study was to detect whether sea hares show different patterns of activities and locations during the day and the night, the results were pooled to four 6 hr time blocks. This was done to refine the detection and to obtain results comparable to those of Carefoot (1989). Times blocks were: (1) from 00:00 to 06:00; (2) 06:00 to 12:00; (3) 12:00 to 18:00; and (4) 18:00 to 24:00. The experiment lasted 20 days.

Statistical analyses of the results were made after square root transformation of the percentages, and included two-way ANOVA between day and night and between treatments and Tukey tests between time blocks and between treatments for each location and for each type of activity. Analyses were performed with Systat 5.04 for Windows (Wilkinson, 1990). Significant differences were declared when $P < 0.05$.

RESULTS

In order to compare general patterns of location and activities between sea hares in the light and those in the shade, data were pooled for each treatment for the whole day (24 hr). This comparison shows that sea hares in group L were significantly more likely to be found buried and under stones and significantly less on the algae. Moreover, they rested more and fed and copulated less than sea hares in group S (Figures 1, 2, $P < 0.0001$).

Dividing the day into four blocks of 6 hours each demonstrated more clearly the circadian patterns of sea hares' location (Figure 1) and activities (Figure 2).

Aplysia oculifera were rarely found buried in this experiment. However most of the records of buried sea hares were collected in group L during daytime (7.1 ± 1.4% and 7.1 ± 1.8% from 06:00 to 12:00 hr and 12:00 to 18:00 hr, respectively). At these time blocks they burrowed significantly more than during night hours (0% during the night), and significantly more than the sea hares in group S (1.8 ± 0.7% between 18:00 to 24:00 h and 0% in the other time blocks).

The preferred location of resting sea hares was under the stone; sea hares were found under stones mainly in group L during the day (58.6 ± 3.4% from 06:00 to 12:00 hr and 56.4 ± 2.3% from 12:00 to 18:00 hr), significantly more than in the same group at night (less than 5.8%) and significantly more than in group S during all 24 hr (2.1 ± 1.4% between 06:00 and 12:00 hr and 0% in the rest of the day).

Sea hares were found on the substrate during all times and activities. In group L, 35.0 ± 2.0% of the sea hares were on the substrate between 00:00 and 06:00 hr, significantly more than at all other times and more than group S which spent only about 25% on the substrate.

Sea hares were usually located on the algae while feeding or copulating. In group S no significant difference between the four time blocks was detected (values ranged between 69.6 and 77.7%). These values were similar to those of group L during the night (61.5 ± 1.4 and 71.9 ± 2.0% from 18:00 to 24:00 hr and from 00:00 to 06:00 hr, respectively). However, during the day, only 11.8 ± 2.6% (06:00 to 12:00 hr) and 17.9 ± 1.6% (12:00 to 18:00 hr) of the sea hares in group L were found on the algae, significantly less than the above.

Patterns of activities within the groups were somewhat more complex. Sea hares were observed resting mostly in group L during the day (more than 60%), significantly more than in the same group at night (about 3%), and more than in group S (< 5.9% during day and night).

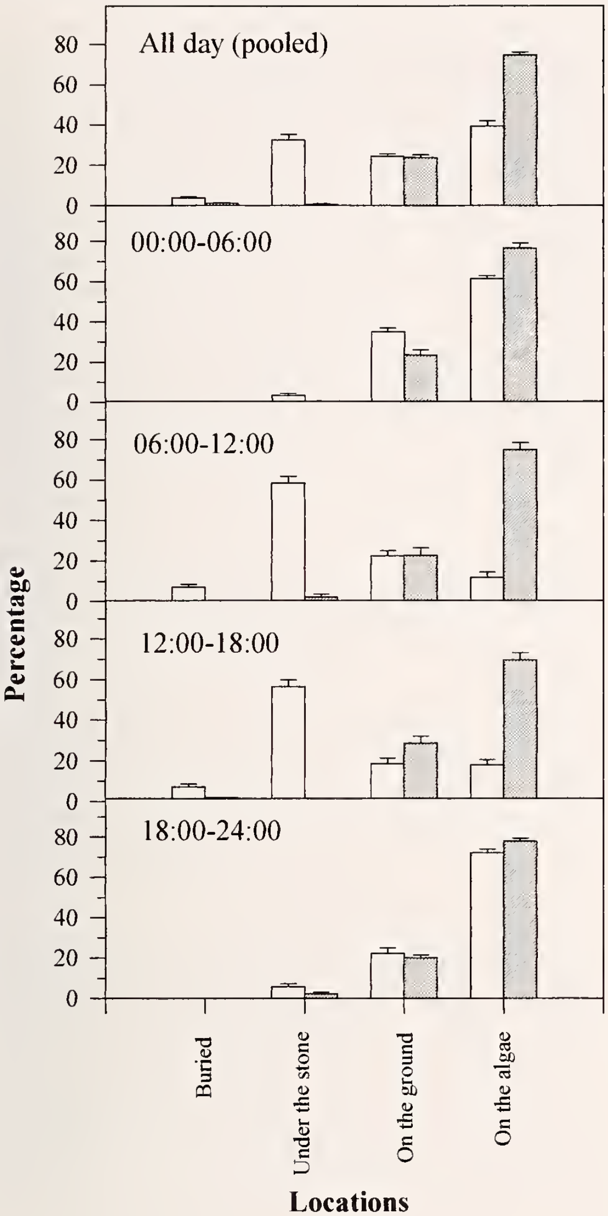

Figure 1

Locations of sea hares *Aplysia oculifera* from the Gulf of Eilat, Red Sea, under natural photoperiod (group L, clear bars) and in the shade (group S, shaded bars) in the aquaria at four time blocks of the day. Bars indicate mean ± SD percentage of sea hares in each group in each location.

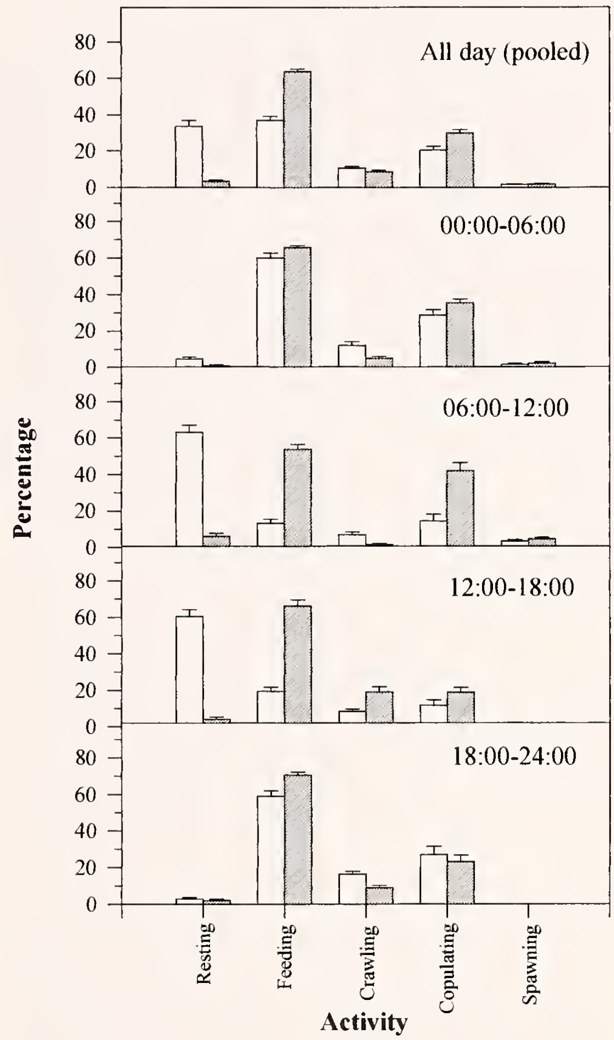

Figure 2

Activities of sea hares, *Aplysia oculifera* from the Gulf of Eilat, Red Sea, under natural photoperiod (group L, clear bars) and in the shade (group S, shaded bars) in the aquaria at four time blocks of the day. Bars indicate mean ± SD percentage of sea hares in each group in each activity.

Feeding showed almost the opposite pattern. In group L only 13.2 ± 2.1% (06:00–12:00 hr) and 19.3 ± 2.2% (12:00–18:00 hr) were found feeding during the day, significantly less than during the night (58.8 ± 3.1 [18:00–24:00 hr] and 60.0 ± 2.8% [00:00–06:00 hr]). In group S significantly higher percentages of feeding sea hares were recorded throughout the 24 hr than in group L during daytime. Percentages of feeding sea hares were similar in both groups during the night. From 06:00–12:00 hr only 53.8 ± 2.6% of the sea hares in group S were feeding (significantly less than during 18:00–06:00 hr in both groups) and 70.4 ± 1.5% between 18:00–24:00 hr, significantly more than all other time blocks in both groups.

Crawling activity was sometimes observed coupled with some other activity (feeding, copulating, or spawning). It did not show a distinct nocturnal pattern. In group L, crawling was observed significantly more during the night (16.2 ± 1.7% at 18:00–24:00 and 11.9 ± 2.1% at 00:00–06:00) than in the daytime (6.8 ± 1.4% at 06:00–

12:00 and 8.2 ± 1.3% at 12:00–18:00). In group S crawling was observed most frequently at 12:00–18:00 (18.9 ± 2.8%), significantly more than at night (8.8 ± 1.7% and 5.0 ± 1.0% at 18:00–24:00 hr and 00:00–06:00 hr, respectively). During the 06:00–12:00 time block, sea hares from group S crawled significantly less than in all other time blocks (1.4 ± 0.7%).

Sea hares in group L showed significant differences between day and night in the frequency of copulation. During the daytime, only 14.3 ± 3.8% and 11.4 ± 3.0% (at 06:00–12:00 hr and 12:00–18:00 hr, respectively) were copulating, significantly lower than at night (29.9 ± 1.9% and 28.8 ± 2.7% at 18:00–24:00 hr and 00:00–06:00 hr respectively). In group S copulation activity was similarly high during the night and the first part of the day (35.4 ± 2.2%, 42.1 ± 4.3% and 23.1 ± 3.5% at 00:00–06:00 hr, 06:00–12:00 hr and 18:00–24:00 hr, respectively) and significantly lower in the second part of daytime (18.6 ± 2.7% at 12:00–18:00 hr).

Unlike all other activities, sea hares in both groups spawned only at the second part of the night (00:00–06:00 hr, 1.5 ± 0.7% and 2.3 ± 0.8% in group L and group S, respectively) and the first part of the day (06:00–12:00 hr, 3.2 ± 0.9% and 4.5 ± 0.9% in group L and group S, respectively). Group L continued to spawn in the second part of the day (12:00–18:00 hr, 1.4 ± 0.7%), significantly less than in the other time blocks. During the 18:00–24:00 hr time block, no spawning was observed.

DISCUSSION

The main finding of this study is that *A. oculifera* demonstrates a nocturnal activity pattern only when exposed to direct sunlight during the day. It has been clearly shown that when under direct sunlight *A. oculifera* was quiescent during the day and active at night. When under the shade, exposed to indirect light of natural photoperiod, where there were, at least, potential light-related cues as to the time of the day, they showed no pattern of nocturnalism, and were active throughout the whole day and night.

Plaut et al. (1996) showed that under shade *A. oculifera* grew somewhat faster than under direct sunlight. The fact that more than 60% of the *A. oculifera* individuals under shade were observed feeding throughout the day (Figure 2) makes it doubtful that there is any energetic advantage for *A. oculifera* being quiescent for more than about 30% of the day, as was suggested by Carefoot & Taylor (1988). Moreover, under direct sunlight, more than 70% of the sea hares were inactive and were located in the shade (under stone or buried). About 20% of the sea hares were on the algae, possibly in partial shade. In addition, *A. oculifera* under natural photoperiod of direct sunlight grew at a rate somewhat slower, although not significantly different than under shade (Plaut et al., 1996). The slower growth rate of sea hares, coupled with greater egg production under direct sunlight (Plaut et al., 1996) suggests that they may have been feeding less and were under suboptimal conditions of restricted feeding (Plaut et al., 1996). Thus, no energetic advantage of being quiescent for part of the day can be claimed in this case.

The fact that sea hares under shade showed neither a nocturnal nor a diurnal pattern of activity suggests that the cue for nocturnalism of sea hares in their natural habitat is exogenous. It seems that in this case, as reported previously (Jacklet, 1976; Carefoot & Taylor, 1988; Carefoot, 1989) direct sunlight is the cue for this pattern. Sunlight containing ultraviolet wavelengths has been found to directly harm tropical shallow-water organisms. Damage may include decrease in respiration, growth rates, and calcification rates, and death (Shick et al., 1996). Indirectly, ultraviolet radiation may damage shallow-water organisms via photochemical reactions that produce reactive oxygen molecules like H_2O_2 (Shick et al., 1996). The natural concentration of stratospheric ozone, generally less near the Equator than at higher latitudes (Cutchis, 1982), together with the lower solar zenith angle in tropical regions, means that the tropics receive more ultraviolet radiation. Thus, tropical ecosystems have an evolutionary history of exposure to high fluxes of ultraviolet radiation (Green et al., 1974; Frederick et al., 1989). The transparency of tropical seawater allows penetration of ultraviolet radiation in shallow-water habitats (Kirk, 1994). Thus, if ultraviolet radiation may harm sea hares, as it does other organisms in tropical shallow water, an evolution toward avoidance of being exposed to this radiation is highly probable.

Carefoot & Taylor (1988) and Carefoot (1989) considered avoidance of light, particularly ultraviolet wavelengths, as a possible advantage of nocturnalism in shallow-water populations of tropical *Aplysia.* However, they raised three arguments against this hypothesis. First, "light is unlikely to have operated as a primary force leading to the evolution of nocturnalism." Second, *A. dactylomela,* as *A. oculifera* (Plaut, 1993) makes no obvious attempt to protect its eggs from the sun's rays. Finally, many other species of tropical and subtropical sea hares are active during the day in shallow water.

As for Carefoot & Taylor's (1988) first argument, it is a general statement, not supported by any explanation and thus it remains unclear.

Regarding the argument about unprotected egg masses, *A. oculifera,* like other sea hares, lays its egg masses during day and night (Figure 2) wherever it happens to be located while spawning, either exposed to (Carefoot & Taylor, 1988) or hidden from sunlight (Plaut, personal observation). Rawlings (1996) stated that many benthic marine invertebrates, living in tidal and subtidal habitats, including Mollusca, shield their embryos from direct exposure to ultraviolet radiation by a capsule wall which effectively filters ultraviolet radiation, as in the caenogastropod, *Nucella emarginata* (Deshayes, 1839). Recently, Carefoot et al. (1998) also suggested possible UV protection in eggs of the sea hare, *A. dactylomela.* This may be also the case in the egg masses of *Aplysia oculifera,* indicating evolutionary adaptation of UV avoidance.

The fact that there are populations of sea hares that do not show a nocturnal pattern of activity should be tested for each population individually. Plaut (1993) reported diurnal activity of *A. oculifera* in several cases, in all of which sea hares were, at least partially, protected from direct sunlight, either by high amounts of algae, or by being in relatively deep water (> 2 m). The same may apply to other populations of sea hares observed to be active diurnally, thus strengthening the assumption that nocturnalism in sea hares is a form of an opportunistic behavior, directly aimed at avoiding exposure to ultraviolet radiation.

The only activity that showed a constant circadian pattern in both groups was spawning, which occurred only between midnight and noon. This pattern may be related to avoidance of egg predators before the egg capsules are fully developed. However, results are insufficient to examine this hypothesis.

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